RECEIVED
CENTRAL FAX CENTER
NOV 22 2004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Att. Docket: FISHMAN=4
In re patent application of:
Pnina FISHMAN
Appln. Serial No. 09/700,751
J. Young
Filed: January 4, 2001
Group Art Unit: 1623
Examiner:
Washington, D.C.
For: PHARMACEUTICAL COMPOSITIONS COMPRISING AN ADENOSINE RECEPTOR -

DECLARATION
under Rule 132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir,

I, Gervais Neliat, Ph.D. of CEREP, BP 1 - 86600 Celle l'Evescault, France, hereby declare:

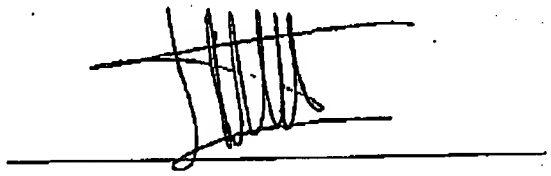
1. I am a Principal Scientist, Pharmacology, in CEREP.

2. Within my capacity in CEREP I was requested to perform the work described in the attached Report (Study Number 8842). As the Study Director, I do attest to the accuracy and completeness of the experimentation described in the Report, which was either performed by me or under my direct supervision.

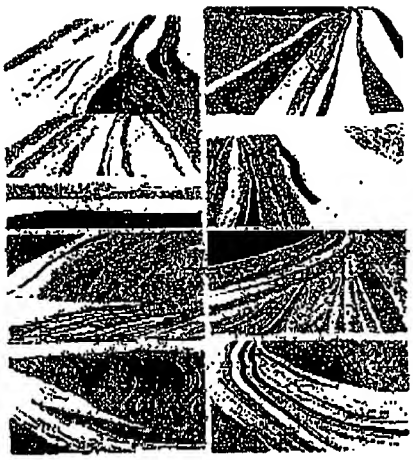
3. The undersigned declares further that all

statements made herein are to the best of his knowledge true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: October 25, 2004



Name: Gervais Neliat, Ph.D.



Cerep

STUDY NUMBER 8842
FINAL REPORT

In Vitro Pharmacology: Human Adenosine Receptors - Study of 6-(γ,γ-Dimethylallylamino) Purine Riboside -

Paris
128 av. Danton
92500 Rueil-Malmaison
FRANCE
tel. +33 (0) 55 94 84 00
fax +33 (0) 55 94 84 10

Poitiers
le Parc Empereur
86600 Celle-L'Evescault
FRANCE
tel. +33 (0)5 49 89 30 00
fax +33 (0)5 49 43 ? 70

Seattle
53 8 NE 95th Street
Redmond WA 98052
USA
tel. +1 (425) 895 8666
fax +1 (425) 895 8668

Tokyo
11th Floor Yuraku-cho Building
1-10-1 Yurakucho Chiyoda-ku
Tokyo 100-0006
JAPAN
tel. +81 (0)3 52 ? 2 2502
fax +81 (0)3 52 ? 2 2493

www.cerep.com
customer_support@cerep.fr

Gervais NELIAT, Ph.D.

Cerep
Le Bois l'Evêque
B.P.1
86 600 Celle l'Evescault
France
www.cerep.com
Tel: 33 (0)5.49.89.30.00
Fax: 33 (0)5.49.43.21.70

Ref.: Final Report 8842/CD

STUDY NUMBER 8842

In Vitro Pharmacology: Human Adenosine Receptors - Study of 6-(γ,γ-Dimethylallylamino) Purine Riboside

Study Sponsor:	CAN-FITE BIOPHARMA
Attention:	Dr. Sara BAR-YEHUDA
Address:	10 Baraekt St., Kiryat Matalon PETACAH TIKVA 49160 ISRAEL
Study Director:	Gervais NELIAT, Ph.D.
Testing Facility:	Cerep Le Bois l'Evêque - B.P. 1 - 86600 CELLE L'EVESCAULT. FRANCE
Study Period:	From August 31, 2004 to September 10, 2004
Report Version:	1
Report Date:	September 14, 2004

STUDY DIRECTOR

Gervais NELIAT, Ph.D.
Principal Scientist, Pharmacology

Date

CONTENTS

1. PURPOSE OF THE STUDY

The purpose of this study was to investigate the effects of 6-(γ,γ-Dimethylallylamino) purine riboside in the *in vitro* human adenosine receptor binding assays.

2. MATERIALS AND METHODS

2.1. *IN VITRO* PHARMACOLOGY: Binding Assays

2.1.1. General Procedures

A_1 *(h)*	human recombinant (CHO cells)	DPCPX	Townsend-Nicholson and Schofield (1994)
A_1 *(h)* (agonist site)	human recombinant (CHO cells)	CPA	Rivkees et al. (1995)
A_{2A} *(h)*	human recombinant (HEK-293 cells)	NECA	Luthin et al. (1995)
A_{2B} *(h)*	human recombinant (HEK-293 cells)	NECA	Stehle et al. (1992)
A_3 *(h)*	human recombinant (HEK-293 cells)	IB-MECA	Salvatore et al. (1993)

2.1.2. Experimental Conditions

A_1 *(h)*	[^{3}H]DPCPX	1 nM	DPCPX (1 µM)	60 min./22°C	Scintillation counting
A_1 *(h)* (agonist site)	[^{3}H]CCPA	1 nM	CPA (10 µM)	60 min/22°C	Scintillation counting
A_{2A} *(h)*	[^{3}H]CGS 21680	6 nM	NECA (10 µM)	90 min./22°C	Scintillation counting
A_{2B} *(h)*	[^{3}H]MRS 1754	0.5 nM	NECA (100 µM)	120 min./22°C	Scintillation counting
A_3 *(h)*	[^{125}I]AB-MECA	0.1 nM	IB-MECA (1 µM)	90 min./22°C	Scintillation counting

2.1.3. Analysis and Expression of Results

The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand.

The results are expressed as a percent of control specific binding obtained in the presence of the test compound.
Individual and mean values are presented in the results section.

The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (n_H) were determined by non-linear regression analysis of the competition curves using Hill equation curve fitting.
The inhibition constants (K_i) were calculated from the Cheng Prusoff equation ($K_i = IC_{50}/(1+(L/K_D))$, where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor).

3. COMPOUNDS

3.1. Test Compound

From: SIGMA-ALDRICH

8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	D7257	059F0633	335.37	1.E-02 M DMSO	1.E-04 M H2O

M.W.: Molecular Weight

3.2. Reference Compounds

In each experiment, the respective reference compound was tested concurrently with the test compound in order to assess the assay suitability. It was tested at several concentrations (for IC_{50} value determination), and the data were compared with historical values determined at Cerep. The assay was rendered valid if the suitability criteria were met, in accordance with the corresponding Standard Operating Procedure.

4. RESULTS

The IC_{50} and K_i values determined for 6-(γ,γ-Dimethylallylamino) purine riboside are indicated in table 1 - 1.
The corresponding competition curves obtained with the test compound are shown in figures 1 to 5.
The individual data obtained with the test compound are reported in table 1 - 2.

The IC_{50} and K_i values for each reference compound are indicated in table 1 - 3. Each is within accepted limits of the historic average ± 0.5 log units.

Table 1 -1

IC_{50} Determination: Summary Results

A_1 *(h)* 8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-05	6.3E-06	*0.4*
A_1 *(h)* (agonist site) 8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	2.5E-07	1.0E-07	*1.0*
A_{2A} *(h)* 8842-1	6-(γ,γ-Dimethylallylamino) purine riboside			> 1.0E-05
A_{2B} *(h)* 8842-1	6-(γ,γ-Dimethylallylamino) purine riboside			N.C.
A_3 *(h)* 8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	7.4E-08	5.1E-08	*0.9*

> Conc. : Above the highest test concentration. IC50 value is above the highest tested concentration. Dose response curve has an inhibitory shape with less than 50 % inhibition at the highest tested concentration

N.C. : Not calculable. IC50 value is not calculable because of less than 25% inhibition at the highest tested concentration.

COMPETITION CURVE OBTAINED WITH COMPOUND
6-(γ,γ-Dimethylallylamino) purine riboside
AT THE HUMAN A1 RECEPTOR

IC50 = 1.0E-05 M
nH = 0.4

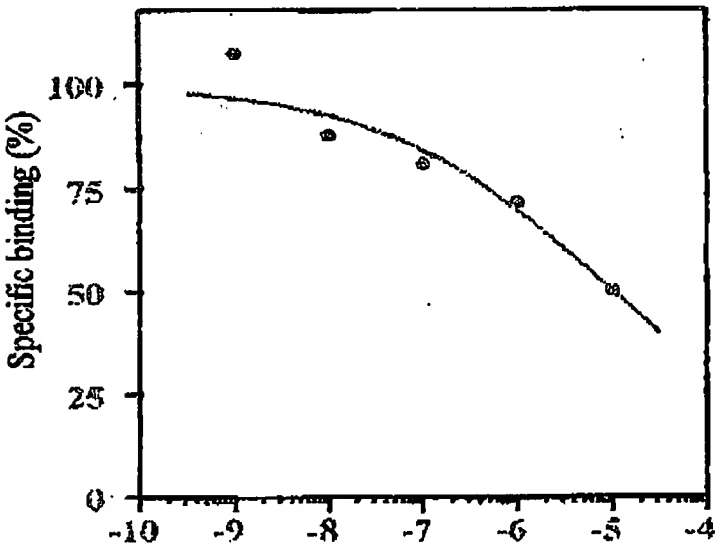


Figure 1

Version 1
September 14, 2004

COMPETITION CURVE OBTAINED WITH COMPOUND
6-(γ,γ-Dimethylallylamino) purine riboside
AT THE AGONIST SITE OF THE HUMAN A1 RECEPTOR

IC50 = 2.5E-07 M
nH = 1.0

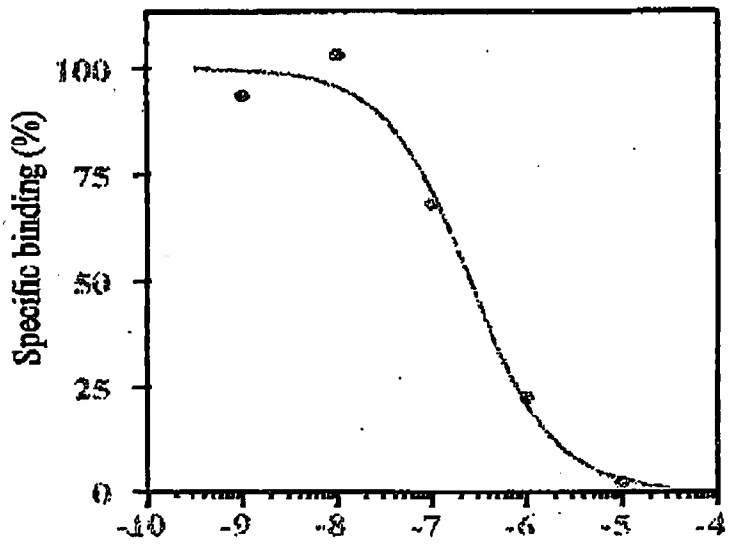


Figure 2

COMPETITION CURVE OBTAINED WITH COMPOUND
6-(γ,γ-Dimethylallylamino) purine riboside
AT THE HUMAN A2A RECEPTOR

IC50 > 1.0E-05 M

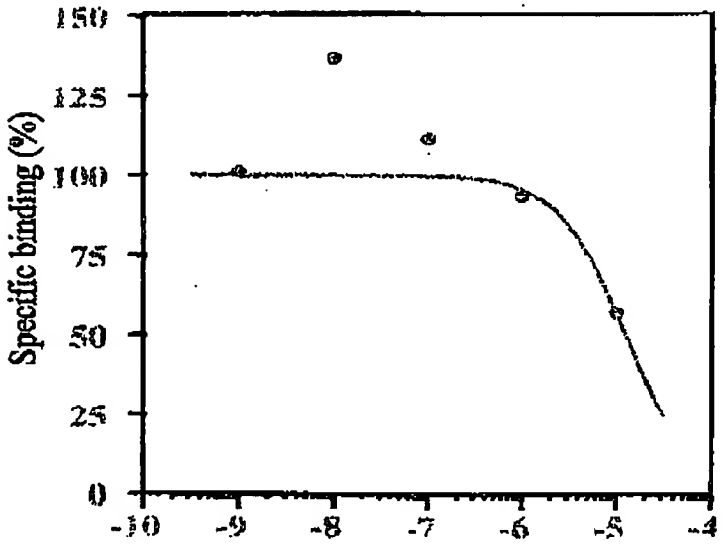


Figure 3

COMPETITION CURVE OBTAINED WITH COMPOUND
6-(γ,γ-Dimethylallylamino) purine riboside
AT THE HUMAN A2B RECEPTOR

IC50 not calculable

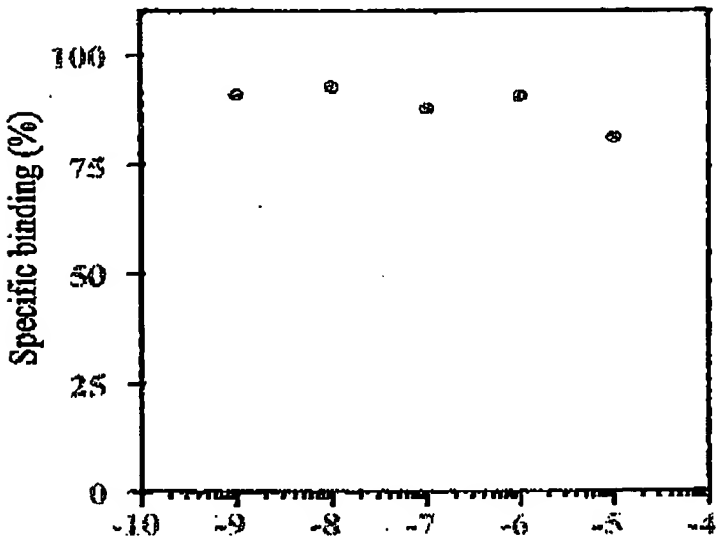


Figure 4

COMPETITION CURVE OBTAINED WITH COMPOUND
6-(γ,γ-Dimethylallylamino) purine riboside
AT THE HUMAN A3 RECEPTOR

IC50 = 7.4E-08 M
nH = 0.9

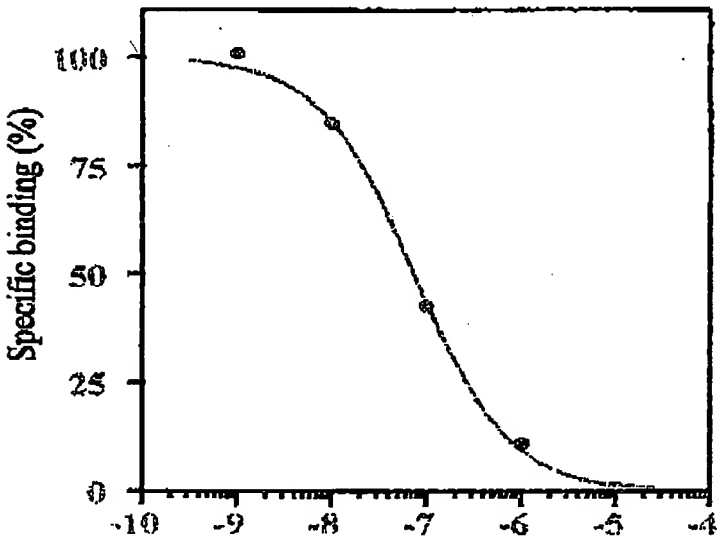


Figure 5

Table 1 - 2

IC_{50} Determination : Individual Data

A_1 *(h)*						
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-09	114.3	100.4	107.3	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-08	89.3	86.2	87.8	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-07	82.0	79.9	80.9	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-06	71.0	72.3	71.6	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-05	54.4	45.5	50.0	
A_1 *(h)* (agonist site)						
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-09	136.1	93.5	93.5	{}
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-08	116.6	89.7	103.2	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-07	77.9	58.0	68.0	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-06	34.3	10.9	22.6	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-05	7.5	-3.1	2.2	
A_{2A} *(h)*						
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-09	103.3	98.8	101.1	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-08	152.2	121.8	137.0	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-07	107.3	115.6	111.4	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-06	93.4	143.7	93.4	{}
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-05	54.5	59.0	56.7	
A_{2B} *(h)*						
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-09	89.6	92.3	90.9	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-08	91.4	94.0	92.7	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-07	87.1	88.3	87.7	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-06	83.6	97.4	90.5	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-05	79.8	82.3	81.0	
A_3 *(h)*						
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-09	97.5	104.5	101.0	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-08	85.0	84.4	84.7	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-07	39.3	46.2	42.8	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-06	12.6	9.2	10.9	
8842-1	6-(γ,γ-Dimethylallylamino) purine riboside	1.0E-05	0.4	1.7	1.1	

{} That replicate was excluded from the calculation

Table 1 - 3

Reference Compound Data

A_1 *(h)* DPCPX	2.4E-08	1.5E-08	*1.3*
A_1 *(h)* (agonist site) CPA	3.9E-09	1.6E-09	*1.1*
A_{2A} *(h)* NECA	5.6E-08	4.6E-08	*1.2*
A_{2B} *(h)* NECA	3.4E-07	3.0E-07	*0.8*
A_3 *(h)* IB-MECA	1.1E-09	7.5E-10	*0.9*

5. BIBLIOGRAPHY

LUTHIN, D.R., OLSSON, R.A., THOMPSON, R.D., SAWMILLER, D.R. and LINDEN, J. (1995)
Characterization of two affinity states of adenosine A_{2a} receptors with a new radioligand, 2-[2-(4-amino-3-[^{125}I]iodophenyl)ethylamino]adenosine.
Mol. Pharmacol., 47 : 307-313.

RIVKEES, S.A., LASBURY, M.E. and BARBHAIA, H. (1995)
Identification of domains of the human A_1 adenosine receptor that are important for binding receptor subtype-selective ligands using chimeric A_1/A_{2a} adenosine receptors.
J. Biol. Chem., 270 : 20485-20490.

SALVATORE, C.A., JACOBSON, M.A., TAYLOR, H.E., LINDEN, J. and JOHNSON, R.G. (1993)
Molecular cloning and characterization of the human A_3 adenosine receptor.
Proc. Natl. Acad. Sci. USA, 90 : 10365-10369.

STEHLE, J.H., RIVKEES, S.A., LEE, J.J., WEAVER, D.R., DEEDS, J.D. and REPPERT, S.M. (1992)
Molecular cloning and expression of the cDNA for a novel A_2-adenosine receptor subtype.
Mol. Endocrinol., 6 : 384-393.

TOWNSEND-NICHOLSON, A. and SCHOFIELD, P.R. (1994)
A threonine residue in the seventh transmembrane domain of the human A_1 adenosine receptor mediates specific agonist binding.
J. Biol. Chem., 269 : 2373-2376.

6. STORAGE AND RETENTION OF RECORDS

All documents generated during the performance of the study (raw data, various recordings such as QA audit reports, an original of the study report, study plan...) will be stored for a 10-year period in Cerep's archive rooms after achievement of the study. Only Cerep's authorized employees shall have access to the archives.

The original final report provided to the sponsor will be kept by the sponsor under its sole responsibility.

7. QUALITY ASSURANCE STATEMENT

The following audits were performed on this study:

	CALENDAR
Audit of Raw Data	For each assay
Audit of the Final Report	

Audit reports were established for each audit performed.
Audit report of the study report was transmitted to the Study Director for approval.

I certify that results presented in this report were generated using the materials and methods mentioned and that these results accurately reflect the Raw Data.

Quality Unit

Version 1
September 14, 2004